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# The effects of endotoxaemia on protein metabolism in skeletal muscle and liver of fed and fasted rats

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The response of muscle and liver protein metabolism to either a single or three successive daily injections of an endotoxin (*Escherichia coli* lipopolysaccharide, serotype 0127 B8; 1 mg/ml, 0.3 mg/100 g body wt.) was studied *in vivo* in the fed rat, and at 24 and 30 h after endotoxin treatment during fasting. In the fed rats there was a catabolic response in muscle, owing to a 60–100% increase in muscle protein degradation rate, and a 52% fall in the synthesis rate. Although there was a 20% decrease in food intake, the decrease in protein synthesis was to some extent independent of this, since rats treated with endotoxin and fasted also showed a lower rate of muscle protein synthesis, which was in excess of the decrease caused by fasting alone. The mechanism of this decreased protein synthesis involved decreased translational activity, since in both fed and fasted rats there was a decreased rate of synthesis per unit of RNA. This occurred despite the fact that insulin concentrations were either maintained or increased, in the fasted rats, to those observed in fed rats. In the liver total protein mass was increased in the fed rats by 16% at 24 h, and the fractional synthesis rate at that time was increased by 35%. In rats fasted after endotoxin treatment the liver protein mass was not decreased as it was in the control fasted rats, and the fractional synthesis rate was increased by 22%. In both cases the increased synthesis rate reflected an elevated hepatic RNA concentration. The extent of this increase in hepatic protein synthesis was sufficient at one point to compensate for the fall in estimated muscle protein synthesis, so that the sum total in the two tissues was maintained.

## INTRODUCTION

The metabolic response to infection, sepsis or trauma includes marked changes in protein metabolism. In the whole body there is an increase in the overall rate of protein turnover (Garlick *et al.*, 1980*a*; Tomkins *et al.*, 1983). Part of this increased turnover is thought to be associated with increased protein synthesis in the liver as part of the acute-phase response, and liver protein mass has been shown to be increased (Yang *et al.*, 1983). In contrast with these changes in the liver, there is a marked overall loss of body protein, much of which originates from skeletal muscle (Beisel, 1977*a*; Powanda, 1977), but the mechanism of this loss is not understood. Furthermore, the design of appropriate therapy depends on the correct diagnosis of the mechanism of this wasting. Although several reports indicate increased muscle protein degradation, there is no consensus as to the changes in muscle protein synthesis, which is reported to be well maintained (Long *et al.*, 1981; Baracos *et al.*, 1983; Clowes *et al.*, 1983; Sobrado *et al.*, 1983; Yang *et al.*, 1983), increased (Pomposelli *et al.*, 1985), or decreased (Klasing & Austic, 1984; Wan *et al.*, 1985). The maintenance of normal rates of protein synthesis in infection would be uncharacteristic, since it is known that muscle protein synthesis is very sensitive to a wide range of insults (Millward *et al.*, 1976), and in all wasting states where direct measurements have been made it has been shown to be depressed (Rennie *et al.*, 1983). We have therefore investigated the effect of infection on muscle and liver protein turnover, examining the acute response *in vivo* to the *Escherichia coli* lipopolysaccharide (LPS) in both fed and fasted rats. A short report of these measurements has previously been published (Jepson *et al.*, 1985).

## EXPERIMENTAL

### Experiment 1

The effect of the endotoxin in fed rats was studied with an initial examination of the response to a single dose over the first 24 h. Twenty male Sprague–Dawley rats (Olac, Bicester, Oxon, U.K.) (98–115 g) were housed at 24 °C, with a 12 h-light/12 h dark cycle (08:00–20:00 h), and given a purified 18% (w/w) - casein diet *ad libitum*. At 09:30 h one group of ten rats was injected subcutaneously with *E. coli* LPS (serotype 0127 B8 phenol extraction; 1 mg/ml; 0.3 mg/100 g body wt; Sigma, Poole, Dorset, U.K.), and a second control group of ten rats was injected with saline (0.9% NaCl). After 24 h, four rats from each group were killed for measurements of muscle and liver protein synthesis. The remaining six rats in each group were killed for additional measurements of muscle and liver protein content.

In a more detailed study of the time course of the response, rats were given repeated daily injections of the endotoxin over a 3-day period. Some 48 male Sprague–Dawley rats (Charles River, Margate, Kent, U.K.) (81–108 g), housed and fed as above, were randomly allocated to eight groups of six rats. At 09:30 h on day 1, six groups were injected with LPS as above and killed at 6, 12, 24, 30, 48 or 72 h after this first injection. Further injections were given at 24 and 48 h so that animals received either one (6, 12 and 24 h), two (30 h and 48 h)

Abbreviations used: LPS, lipopolysaccharide; $T_3$, tri-iodothyronine.

or three (72 h) injections. Saline-injected control groups of rats were killed at 10:00 h on day 1, and after 72 h on day 3. Food intake was measured daily in the untreated and LPS-treated groups killed on day 3.

**Experiment 2**

To examine the response to the LPS independent from any changes in food intake, 24 male Sprague–Dawley rats (Olac; 86–106 g), housed and fed as above, were randomly allocated to four groups of six rats. Food was withdrawn from all animals at 09:30 h, when two groups were injected with LPS (as above) and two control groups with saline. After 24 h one fasted group and one fasted–LPS-treated group were killed for measurement of muscle and liver protein synthesis, and the remaining two groups were given a second dose of LPS or saline and killed 6 h later.

**Measurement of muscle and liver protein synthesis**

Measurements of skeletal-muscle and liver protein synthesis were made by a modification of the technique described by Garlick *et al.* (1980*b*). Animals were injected intraperitoneally with a large dose of radioactively labelled phenylalanine (150 $\mu$mol of phenylalanine and 20 $\mu$Ci of L-[4-$^3$H]phenylalanine/ml; 2 ml/100 g body wt.), and killed 15 min later by decapitation. Blood was collected from the neck, the muscles (combined gastrocnemius and plantaris in Exp. 1; individual gastrocnemius, plantaris and soleus in Exp. 2) and liver were rapidly removed and frozen in liquid $N_2$. Our measurements (P. C. Bates, P. G. Broadbent, L. Chew & D. J. Millward, unpublished work) made in rats in a variety of nutritional states by using this modified method show that the intraperitoneal route of administration of the large dose of labelled phenylalanine results in a plateau of liver intracellular specific radioactivity from within 1 min until at least 15 min. Thus liver protein synthesis ($K_s$) was calculated as

$$K_s = \frac{S_b}{S_i \cdot t}$$

where $S_b$ and $S_i$ are the specific radioactivities of protein-bound and free intracellular phenylalanine respectively, and $t$ is the time (in days) of the incorporation of label.

In muscle the time course of the increase in the intracellular phenylalanine specific radioactivity was a little slower, since the plateau value was not reached for 3 min. Plateau was maintained for at least 15 min. In several experiments with rats in a variety of nutritional states, the time course of the rise to plateau was similar, and the value of the time integral for the phenylalanine specific radioactivity was 0.9 of that which would have been obtained if plateau labelling was instantaneous. Thus for muscle

$$K_s = \frac{S_b}{0.9\ S_i \cdot t}$$

The protein-synthesis rates for both muscle and liver can therefore be calculated for animals killed at a single time point of 15 min after intraperitoneal administration of the radioisotope, instead of two time points after intravenous administration as in Garlick *et al.* (1980*b*).

Protein and RNA concentrations were determined as described previously (Millward *et al.*, 1974), and the RNA activity (g of protein synthesized/g of RNA per day) was calculated by dividing the fractional synthesis rate by the RNA/protein ratio.

**Determination of muscle protein degradation**

Muscle protein degradation was calculated for Expt. 1 as the difference between synthesis and growth over 24 h intervals.

Growth rate of muscle protein was calculated in one of two ways; either as growth in body weight of individual rats, or as the change in mean muscle protein content of groups of rats. The first method was used where the muscle-protein/body-weight ratios did not change, i.e. in the control groups and the 24 h-treated group, so that muscle-protein and body-weight growth rates are the same, and there is no influence of differences in gut fill or altered body composition between groups. When the muscle-protein/body-weight ratios did change (in the 48 h- and 72 h-treated groups), growth rates could only be calculated by a second method. In this case the best value obtainable takes into account the contribution of variability in initial body weights to the variability of muscle protein content at death by correcting final body weights and tissue protein contents by a factor which was the ratio of the individual to the mean initial body weight. This was 87 g (measured at $t = -24$ h) in Expt. 1 and 98 g (measured at $t = 0$ h) in Expt. 2.

To estimate the mean values of muscle protein synthesis over each 24 h period in control animals, we have in the past assumed that the value measured at 10:00 h is similar to the mean value over a 24 h cycle. To test this assumption, we have measured muscle protein synthesis at 1 and 7 h into the light period (i.e. at 09:00 and 15:00 h), when feeding is minimum, and at 1 h into the dark period (at 21:00 h), when feeding is maximum. Table 1 shows that the extent of the diurnal variation in protein synthesis was small, such that in well-fed rats under these conditions the synthesis rate measured at 09:00 h was close to the weighted mean value for a complete 24 h cycle. Thus in these cases individual values for degradation rate can be calculated (as synthesis − growth), with estimations of mean values and standard deviations.

In the treated rats, where muscle protein synthesis is changing, only group mean synthesis rates can be estimated for each time period. These were calculated as the weighted (for time) mean values of the synthesis rates of the groups killed during each 24 h interval. In these cases only group mean degradation rates with no indication of errors could be estimated.

**Table 1. Diurnal variation in muscle protein synthesis**

Muscle used was combined gastrocnemius and plantaris. Protein synthesis was measured by the large-dose phenylalanine method (Garlick *et al*, 1980*b*). Animals were housed with a 12 h-light/12 h-dark cycle. The light period commenced at 08:00 h, so that the measurements of 09:00 and 15:00 h correspond to 1 and 7 h into the light period, and 21:00 h was 1 h into the dark period. Results are given as means ±S.D. ($n = 4$).

| Time (h) | Synthesis (%/day) |
|---|---|
| 09:00 | 23.8±2.2 |
| 15:00 | 21.6±3.5 |
| 21:00 | 26.2±3.1 |
| Weighted average | 24.5±2.5 |

**Table 2. Effect of endotoxin on muscle and liver protein content, plasma glucose and hormones**

Experimental details are given in the text. Muscle used was combined gastrocnemius and plantaris in Expt. 1, and gastrocnemius in Expt. 2. Results are given as means ± S.D. ($n = 6$). Body weights and tissue protein contents at death were corrected to the same initial body weight as described in the text, to take into account the contribution of variability in initial body weight to the variability of tissue protein content at death. Muscle protein content of the 24 h and 48 h untreated groups (Expt. 1) was calculated from the body weights of the 72 h untreated group at 24 and 48 h, and the muscle-protein/body-weight ratio of the three control groups, as described in the Experimental section. Plasma free $T_3$ was measured in pooled plasma for each group; therefore variability cannot be estimated. *$P < 0.02$ compared with 0 h untreated group (Expt. 1), or with respective 24 h- or 30 h-fasted group (Expt. 2).

| Expt. no. | Time (h) | Treatment | Body wt. (g) | Muscle protein content (mg) | Liver protein content (mg) | Plasma glucose (mg/100 ml) | Plasma insulin ($\mu$ units/ml) | Plasma total $T_3$ (ng/ml) | Plasma free $T_3$ (pg/ml) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0 | Untreated | 94±1.1 | 68.1±1.6 | 656±77 | 178±5.4 | 30.8±4.7 | 1.04±0.13 | 7.9 |
| | 6 | LPS | 95±1.1 | 69.2±4.3 | 614±34 | 148±6.0 | 32.1±5.1 | 1.16±0.14 | 8.8 |
| | 12 | LPS | 95±1.0 | 68.9±1.1 | 592±43 | 159±8.5 | 39.4±5.7 | 0.76±0.13* | 7.2 |
| | 24 | Untreated | 102±1.7 | 75.1±3.4 | | | | | |
| | 24 | LPS | 95±2.1 | 69.2±5.1 | 762±59* | 158±9.1 | 34.3±6.6 | 0.68±0.07* | 4.8 |
| | 30 | LPS | 93±3.7 | 66.7±3.6 | 713±60 | 159±5.7 | 31.8±4.9 | 0.70±0.10* | 6.4 |
| | 48 | Untreated | 110±2.0 | 81.0±3.7 | | | | | |
| | 48 | LPS | 104±2.3 | 67.6±2.4 | 686±37 | 156±4.1 | 34.7±4.0 | 0.76±0.08* | 7.9 |
| | 72 | Untreated | 118±2.9 | 88.0±5.6* | 801±67* | 151±14.8 | 31.6±4.8 | 1.23±0.13 | 9.0 |
| | 72 | LPS | 110±1.3 | 75.0±2.2* | 688±16 | 162±7.1 | 34.2±3.3 | 0.77±0.05* | 7.7 |
| 2 | 24 | Fasted | 89±0.8 | 70.5±3.8 | 565±32 | | 16.9±8.6 | | |
| | 24 | +LPS | 88±0.6 | 65.9±4.7 | 650±40* | | 29.7±9.6* | | |
| | 30 | Fasted | 89±0.9 | 67.8±3.2 | 561±35 | | 17.3±4.5 | | |
| | 30 | +LPS | 88±0.8 | 63.9±2.8 | 647±34* | | 30.4±13.8 | | |

### Plasma hormone and glucose analysis

Plasma insulin concentration was measured as previously described (Odedra *et al.*, 1982). Plasma free and total $T_3$ concentrations were measured with radioimmunoassay kits (Metachem Diagnostics, Northampton, U.K.). Plasma glucose was assayed with glucose oxidase, by using the GOD-Perid assay kit from Boehringer Corp. (London).

Data are expressed as means ± S.D. Statistical analysis was by one-way analysis of variance, and by Student's *t* test where appropriate.

## RESULTS

The results of the initial experiment were similar to those in the more detailed time-course study, so that only the latter results are tabulated. The effect of LPS on body weights, muscle and liver protein content and plasma glucose, insulin and $T_3$ in the fed and fasted rats is shown in Table 2. The LPS treatment decreased food intakes, but not markedly so. Intake was 11.6 ± 0.5 g/day before the LPS treatment, and fell by 20% on the first day, remained lower (11%) on day 2, but returned to normal after this. These changes in food intake appeared to have little effect on blood glucose concentrations (Table 2), since none of the treated groups exhibited significantly lower values than the mean of the three control groups (154 mg/100 ml). Notwithstanding the relatively minor decrease in food intake, the first dose of LPS caused a complete cessation of whole-body growth after 24 h, which persisted throughout the second day after the second dose. After 48 h, growth recovered, despite the administration of a third dose of LPS at 48 h. In the fasted rats the LPS treatment had little extra catabolic influence as far as body-weight changes were concerned.

The catabolic response was also indicated by the abrupt slowing of muscle growth. In the preliminary experiment the muscle-protein/body-weight ratio was depressed (70.7, cf. 73.9 mg/100 g body wt.), suggesting an actual loss of protein, but in the main experiment this did not appear to occur until after the second dose. This specific catabolic effect was also observed in the fasted rats, since the muscle protein content was decreased (significantly so when the muscle-protein/body-weight ratios are compared). However, after the second dose, in the fed rats LPS resistance developed, so despite the third dose at 48 h the recovery began and there was rapid muscle growth.

In contrast with the catabolic response of muscle, the liver-protein/body-weight ratio was increased by 16% in the first 24 h (Table 2), twice that expected if normal growth occurred, so that the increase in liver protein at 24 h was a specific anabolic response and not simply a lack of any inhibitory effect on the liver. This was also demonstrated in the fasted rats, since the liver protein was significantly increased compared with control groups at both 24 and 30 h.

Insulin concentrations were almost doubled in the preliminary experiment (from 27.8 to 46.1 μunits/ml) and were well maintained throughout the 3-day study (Table 2). In the fasted LPS-treated rats, plasma insulin was increased to values comparable with those in the untreated fed rats. Plasma total $T_3$ was decreased at 12 h onwards in the LPS-treated rats, and remained low even after 72 h, when the rats were rapidly growing. Free $T_3$ was also decreased, but the time course of the changes was different, since they were lowest at 24 and 30 h and normal at the other time points.

Protein synthesis was decreased in muscle and increased in liver of the fed rats after the endotoxin treatment (Table 3). In the preliminary experiment, muscle protein synthesis was decreased by 30% after 24 h, whereas in the time-course study the decrease was 40% after 24 h. Whether there was an earlier effect is not completely clear, since, although the rate had fallen by 13% by 12 h, this was not a significant change. However, the measurements of diurnal changes (Table 1) show that the rate at 21:00 h was increased somewhat compared

**Table 3. Effect of endotoxin on protein synthesis in skeletal muscle and liver of fed rats (Expt. 1)**

Experimental details are given in the text. Results are given as means ± S.D. ($n = 6$). Muscle used was combined gastrocnemius and plantaris. *$P < 0.05$ compared with 0 h untreated group.

| Tissue | Time (h) | Treatment | Protein synthesis (%/day) | Protein synthesis (g/day per g of RNA) | RNA concn. (mg/g of protein) |
|---|---|---|---|---|---|
| Muscle | 0 | Untreated | 17.5 ± 0.5 | 11.3 ± 1.1 | 15.3 ± 0.9 |
| | 6 | LPS | 16.1 ± 1.5 | 10.2 ± 0.9 | 15.9 ± 1.4 |
| | 12 | LPS | 15.2 ± 2.1 | 10.9 ± 1.5 | 14.0 ± 1.5 |
| | 24 | LPS | 10.3 ± 1.7* | 6.9 ± 1.2* | 15.1 ± 1.2 |
| | 30 | LPS | 8.4 ± 1.5* | 5.8 ± 1.1* | 13.8 ± 2.1 |
| | 48 | LPS | 11.2 ± 1.4* | 8.8 ± 1.5* | 12.7 ± 0.7* |
| | 72 | LPS | 13.1 ± 1.1* | 10.7 ± 1.2 | 12.6 ± 1.7* |
| | 72 | Untreated | 15.9 ± 1.3 | 11.3 ± 1.3 | 14.4 ± 1.0 |
| Liver | 0 | Untreated | 110.5 ± 15.5 | 15.6 ± 2.8 | 71.2 ± 2.7 |
| | 6 | LPS | 117.2 ± 15.4 | 15.3 ± 2.0 | 76.8 ± 3.8* |
| | 12 | LPS | 126.9 ± 21.7 | 15.9 ± 2.5 | 79.7 ± 2.0* |
| | 24 | LPS | 149.4 ± 21.0* | 17.5 ± 3.4 | 86.0 ± 5.2* |
| | 30 | LPS | 141.7 ± 7.7* | 16.5 ± 1.4 | 85.9 ± 4.8* |
| | 48 | LPS | 123.0 ± 18.7 | 15.8 ± 2.6 | 78.0 ± 3.4* |
| | 72 | LPS | 101.5 ± 7.3 | 14.1 ± 1.4 | 73.8 ± 3.1 |
| | 72 | Untreated | 114.1 ± 23.2 | 16.7 ± 2.5 | 71.3 ± 6.1 |

with that at 09:00 h, suggesting that an effect of the endotoxin on muscle protein synthesis is manifest within the first 12 h. After the second dose, at 30 h, the rate fell further, to 48% of the initial control rate. Thereafter the synthesis rate recovered, and continued to improve despite the third dose of LPS.

The changes in protein synthesis in muscle were mainly due to decreases in the RNA activity, which was significantly decreased below the initial control measurement between 24 and 48 h. RNA concentration showed very little initial change, but was significantly decreased after 48 h.

The fractional rate of liver protein synthesis was increased by one-third after 24 h, with earlier values showing the same trend, and the RNA concentration significantly increased as early as 6 h and at all subsequent times up to 48 h. By 72 h the rate had fallen back to control values. Whether the RNA activity changed was not clear, since, although the value was increased at 24 h, the change was small and not significant.

The influence of these opposite changes in muscle and liver protein synthesis on the rate of protein synthesis in the whole body can be to some extent estimated from the calculated rate of protein synthesis in the total liver and muscle tissue, assuming that the changes in the protein synthesis rate and protein content in the mixed-fibre gastrocnemius muscle were representative of the rate in the whole musculature. These values are indicated in Fig. 1, where it is clear that the fall in protein synthesis in muscle was to a considerable extent matched by the increase in liver. After 24 h the liver protein mass was increased in absolute terms and as a proportion of body weight, and, since the fractional rate of protein synthesis was also increased (by 35%) at this time, the absolute rate of protein synthesis was increased by an even greater extent (57%), and remained significantly elevated at 30 h.

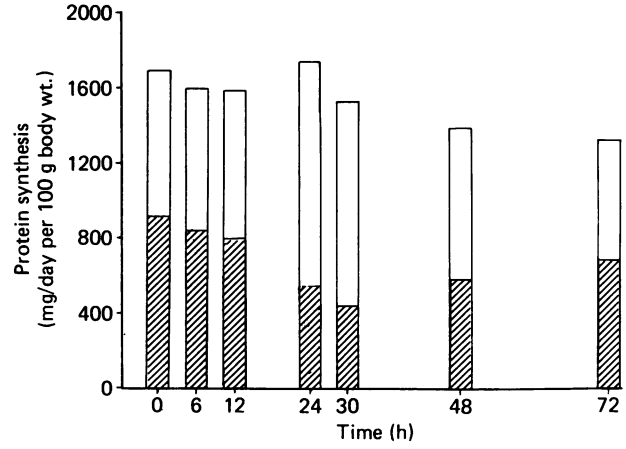


**Fig. 1. Effect of endotoxin on distribution of protein synthesis between skeletal muscle and liver of fed rats**

Experimental details are given in the text. ▨, Muscle protein synthesis; □, liver protein synthesis. The absolute rate of muscle protein synthesis was calculated by multiplication of total muscle protein by the fractional synthesis rate. Total muscle protein was calculated from total muscle weight (from the assumption that muscle constitutes 35% of body weight; Miller, 1969) multiplied by the protein concentration measured in the gastrocnemius and plantaris muscles. The absolute rate of liver protein synthesis was similarly calculated from the liver weight, protein concentration and fractional synthesis rate.

Thus at 24 h the total protein synthesis in muscle and liver was actually increased.

In the experiments on fasted rats, measurements were made on the individual gastrocnemius, plantaris and soleus muscles. Fasting alone caused a decrease in muscle protein-synthesis rate (Table 4), which fell in the

**Table 4. Effect of endotoxin on protein synthesis in skeletal muscle and liver of fasted rats (Expt. 2)**

Experimental details are given in the text. Results are given as means ± S.D. ($n = 6$). *$P < 0.05$ compared with respective 24 h or 30 h control.

| Tissue | Time (h) | Treatment | Protein synthesis (%/day) | Protein synthesis (g/day per g of RNA) | RNA concn. (mg/g of protein) |
|---|---|---|---|---|---|
| Muscle | | | | | |
| Gastrocnemius | 24 | Fasted | 11.3±1.9 | 9.7±1.4 | 11.7±0.8 |
| | | +LPS | 8.5±0.9* | 7.9±0.7* | 10.8±0.8 |
| | 30 | Fasted | 8.9±1.3 | 8.2±1.2 | 10.8±0.5 |
| | | +LPS | 7.2±1.2* | 6.6±0.8* | 11.0±0.8 |
| Plantaris | 24 | Fasted | 12.5±2.5 | 11.7±2.2 | 10.7±0.5 |
| | | +LPS | 8.9±1.5* | 9.3±2.0 | 9.6±1.0* |
| | 30 | Fasted | 9.7±1.6 | 10.5±0.9 | 9.2±1.1 |
| | | +LPS | 7.1±1.4* | 7.5±1.6* | 9.5±0.4 |
| Soleus | 24 | Fasted | 16.6±2.8 | 11.5±1.6 | 14.5±2.0 |
| | | +LPS | 13.6±1.7* | 10.5±2.0 | 13.1±1.4 |
| | 30 | Fasted | 15.3±1.6 | 11.9±1.3 | 12.8±1.2 |
| | | +LPS | 11.1±0.7* | 9.5±1.5* | 12.0±1.5 |
| Liver | 24 | Fasted | 106.3±26.3 | 19.4±4.6 | 54.7±3.6 |
| | | +LPS | 129.6±23.0 | 20.3±2.2 | 63.6±5.2* |
| | 30 | Fasted | 99.5±11.0 | 18.3±1.6 | 54.2±3.6 |
| | | +LPS | 121.8±14.4 | 18.7±2.3 | 65.4±1.4* |

**Table 5. Effect of endotoxin on muscle protein degradation (Expt. 1)**

Experimental details are given in the text. The growth rates were calculated from body-weight gain in those groups where there was no change in the muscle-weight/body-weight ratio, i.e. in the two control groups and the 0–24 h LPS-treated group. In the remaining treated groups there was a change in body composition, and so growth was calculated from mean values for muscle protein content, as described in the text. Protein synthesis in the treated groups is the weighted mean of measurements made during each 24 h interval. Protein degradation was calculated as the difference between these two rates.

| Time (h) | Treatment | Growth rate (%/day) | Synthesis rate (%/day) | Degradation rate (%/day) |
|---|---|---|---|---|
| 0 | Untreated | 8.7±0.5 | 17.6±0.5 | 8.9±0.9 |
| 0–24 | LPS | −0.2±2.6 | 14.5 | 14.7 |
| 24–48 | LPS | −2.3 | 9.7 | 12.0 |
| 48–72 | LPS | 10.4 | 12.2 | 1.8 |
| 72 | Untreated | 7.2±2.3 | 15.9±1.3 | 8.7±3.1 |

gastrocnemius to 11.3%/day after 24 h, and 8.9%/day after 30 h fasting, from 15.6 and 17.5%/day in the combined gastrocnemius and plantaris in the fed rats. There were similar changes in the plantaris. However, the combination of food withdrawal and LPS treatment caused an even greater fall in muscle protein synthesis, which was depressed by 24% in the gastrocnemius, 29% in the plantaris and 18% in the soleus compared with fasted control values 24 h after a single dose of LPS, and was similarly depressed after 30 h. As in the fed rats of Expt. 1, the decrease in synthesis rate was due mainly to a fall in RNA activity, with lesser decreases in the RNA concentration (Table 4).

Comparison of the liver protein content of fasted rats with that of the fed rats of Expt. 1 indicates that fasting induced a fall in the liver protein content, which was not evident in the fasted–LPS-treated rats. Furthermore the fractional rate of protein synthesis in the liver was increased by 22% above the fasted control rate at both 24 and 30 h, attributable to increased RNA content, as in the fed endotoxaemic rats.

The persistence of these changes in protein synthesis in muscle and liver in response to the LPS when the treatment is combined with fasting demonstrates the response to be a direct effect of the LPS rather than an indirect effect via alterations of food intake.

Rates of muscle protein degradation were calculated in Expt. 1 and are shown in Table 5. The rates were similar in the two control groups. In the treated groups degradation was increased by 65% in the first 24 h interval after the LPS (100% in the preliminary experiment), and remained elevated in the subsequent 24 h, after the second dose. After the third dose at 48 h, since protein synthesis was not restored to control values, the substantial growth that occurred after this time did so because of the marked fall in protein degradation. This pattern of response was also observed when degradation was based on a growth-rate value calculated by the alternative method. As explained above for the 0–24 h LPS-treated and control groups, growth rate was calculated from body-weight changes, since muscle-protein/body-weight ratios were constant in these animals. Growth rate calculated for the 0–24 h group from the mean protein contents in Table 2 would have been +2.3%/day, indicating a degradation rate of 12.2%/day, 37% increased above control.

In the fasted rats, although the data do not allow an accurate determination of muscle degradation rate, this appeared to be increased above the value in the untreated fasted group, since the additional fall in protein synthesis (2.3%/day below that of the fasted rat) is insufficient to account for the lower protein content in the treated groups (6.6% at 24 h and 5.9% at 30 h). This indicates that the LPS treatment caused on average additional degradation of 6.2%/day more than that caused by fasting alone.

## DISCUSSION

The rat responds to the sublethal dose of the *E. coli* LPS that we have used with a slowing of growth, muscle wasting and hepatic enlargement. This response is only temporary, and complete resistance to this challenge is achieved within 3 days, as previously described (McCabe, 1980).

Previous studies of the changes in muscle protein synthesis in infection are conflicting. Older studies (Lust, 1966; Young *et al.*, 1968; Powanda *et al.*, 1972) consistently concluded that synthesis was decreased. In contrast, more recent studies have indicated a range of responses, with muscle protein synthesis decreased (Klasing & Austic, 1984; Wan *et al.*, 1985), maintained (Clowes *et al.*, 1983; Baracos *et al.*, 1983; Yang *et al.*, 1983; Sobrado *et al.*, 1983), or even increased (Pomposelli *et al.*, 1985). The absence of a response in incubated muscle (Clowes *et al.*, 1983; Baracos *et al.*, 1983) is not surprising, because the synthesis rate is already in a stepped-down state, particularly when muscles are incubated in the absence of tension (Palmer *et al.*, 1981). In addition, the fact that we did not see any change in the rate of protein synthesis for the first 24 h after a dose of LPS could explain the lack of response in guinea pigs infused with leucocytic pyrogen for only 4 h (Sobrado *et al.*, 1983). However, we cannot explain the increased synthesis rate in bacteraemic rats reported by Pomposelli *et al.* (1985), but that study is difficult to evaluate, since no details are given of the methods used to measure the leucine specific radioactivity. The measurements of muscle protein synthesis reported here support the earlier findings of decreased protein synthesis in infection.

In the fed rats, the fall in muscle protein synthesis may have been at least partly due to the decreased food intake induced by the LPS, but the decreased synthesis in the fasted–LPS-treated rats makes this unlikely. It seems

more likely to be a direct response to LPS rather than to alteration in nutrient supply. The main factor responsible for the regulation of the translational phase of muscle protein synthesis is insulin (Jefferson, 1980; Garlick *et al.*, 1983; Millward *et al.*, 1983), and in the present experiments insulin was either maintained or even increased (in the fasted rats to values oberved in the fed rats), in line with previous reports (Ryan *et al.*, 1974; Buchanan & Filkins, 1976). The observation that decreased RNA activity occurs concurrently with normal or increased insulin implies that the muscle tissue is insulin-resistant, a phenomenon established for glucose metabolism in some (Drobny *et al.*, 1984) but not all studies (Romanosky *et al.*, 1980). Corticosterone, which we have shown to inhibit insulin action on muscle (Odedra *et al.*, 1982; Millward *et al.*, 1983), has been suggested as a mediator of this insulin resistance (Drobny *et al.*, 1984). Furthermore, the overall pattern of response of muscle and liver is to some extent similar to the initial response to treatment with catabolic doses of corticosterone (Odedra *et al.*, 1983). However, our measurements of corticosterone in quietly killed, fed, endotoxaemic rats show only moderate increases: $119 \pm 75$ ng/ml at 24 h, and $158 \pm 62$ ng/ml at 30 h, compared with a control value of $71 \pm 33$ ng/ml. We believe therefore that the increased corticosterone can only be partly responsible for the decreased RNA activity and that other factors, such as the endotoxin itself or a cytokine such as interleukin 1, may well be active, as concluded by Beisel (1977*b*).

One response that could be hormonally mediated is the fall in RNA concentration, which could reflect the lower $T_3$ concentration. Changes in plasma $T_3$ have been shown to induce parallel decreases in muscle RNA (Brown *et al.*, 1981; Brown & Millward, 1983), most likely reflecting decreased rRNA synthesis (Millward *et al.*, 1981). The mechanism for the decrease in the plasma $T_3$ has been shown to be decreased conversion of thyroxine into $T_3$, and increased clearance from the plasma (Goretzki *et al.*, 1983).

Our finding of increased muscle protein degradation is consistent with previous reports (Long *et al.*, 1981; Clowes *et al.*, 1983; Baracos *et al.*, 1983; Yang *et al.*, 1983). Muscle proteolysis often reflects the thyroid-hormone status (Millward *et al.*, 1985), but in the present experiments the increases in muscle proteolysis were clearly unrelated to thyroid hormones, which decreased throughout the experiment. Possibly after the catabolic response to the LPS had ceased (after 48 h), the decreased muscle proteolysis could have reflected the decrease in $T_3$.

Several investigations of the increased proteolysis in infection (Clowes *et al.*, 1983; Baracos *et al.*, 1983) have emphasized the role of cytokines, which are postulated to increase prostaglandin $E_2$, in turn activating lysosomal proteolysis (Ruff & Secrist, 1984; Goldberg *et al.*, 1984). However, other studies with inhibitors of prostaglandin synthesis (Sobrado *et al.*, 1983; Clark *et al.*, 1984; Hulton *et al.*, 1985; Turinsky & Loegering, 1985) have failed to confirm any role for prostaglandins. Indeed, Freund *et al.* (1985) failed to observe any change in prostaglandin release from incubated muscle from septic rats. It is very difficult to account for the decrease in muscle protein synthesis in terms of a mechanism involving prostaglandins. They are reported to increase muscle protein synthesis, at least in the case of prostaglandin $F_{2\alpha}$ (Reeds & Palmer, 1983; Reeds *et al.*, 1985), and in incubated muscle parallel changes in prostaglandins $E_2$ and $F_{2\alpha}$ occur (Reeds & Palmer, 1984). Thus, although any increased prostaglandin synthesis in muscle in response to infection would be consistent with the maintenance or even an increase in muscle protein synthesis, it is not consistent with the decreased muscle protein synthesis that we and others (Wan *et al.*, 1985; Klasing & Austic, 1984) have observed.

In contrast with muscle, hepatic protein synthesis was markedly elevated in LPS treated rats. This response has been observed previously, and includes the increased synthesis of the acute-phase proteins (Beisel, 1977*a*). Part of the response in the fed rats could reflect the increased amino acid supply to the liver (consequent on the block on peripheral uptake) in the same way in which the influx of amino acids after a meal increases liver size and RNA concentration (Millward *et al.*, 1974), although most of the mechanism of this response is a near-total suppression of proteolysis (Poso *et al.*, 1982). However, the fact that hepatic protein content and synthesis were increased in the fasted rats (albeit not to the same extent as in the fed rats) indicates that other mechanisms are operating. Wannemacher *et al.* (1975) and more recent studies (Koj *et al.*, 1984) provide evidence for a direct role of leucocytic pyrogens in the response. Since the changes in liver are very similar to those after treatment with exogenous corticosterone (Odedra *et al.*, 1983), it is likely that corticosterone is integral to the response, as well as the leucocytic pyrogen.

After 24 h, in the fed rats this increase in liver protein synthesis more than compensated for the decrease in muscle, so that the total was actually increased. Since it is likely that protein synthesis is increased in other tissues, such as small intestine (Lust, 1966), spleen, bursa and thymus (Klasing & Austic, 1984), this would also contribute to increases in whole-body protein synthesis, which are seen in human studies. Clearly this is an example of marked changes in the distribution as well as the rate of whole-body protein synthesis.

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